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Chemical constituents of *Lonicera cambodiana* leaves

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***Abstract*—The leaves of *Lonicera cambodiana* was subjected to purified secondary metabolites. Using combination of chromatographic methods, four compounds were isolated including loganin (1), sweroside (2), pinoresinol 4-O-β-D-glucopyranoside (3), and syringaresinol 4-O-β-D-glucopyranoside (4). Their chemical structures were determined by analysis of ESI-MS, NMR spectra, and as well as comparison with reported literature. This is the first chemical study of *L. cambodiana* species.**

Keywords—Lonicera cambodiana; secoiridoid; iridoid; lignan.

I. INTRODUCTION

The *Lonicera* genus has documented around 200 species [1]. Some of *Lonicera* species were cultured as ornamental plants due to their scented flower and some of species produced edible sweet tasting fruits, called honeysuckle such as *L. caerulea*, *L. japonica*. Chemical investigations of honeysuckle fruits have been reported rich of anthocyanins, phenolic acids, flavonoids which possess broad spectrum of biological activities, especially, antioxidant, anti-inflammation, and antimicrobial activity [2-4].

In the course of chemical investigation of *Lonicera* species, this work describes the isolation and chemical structure elucidation of four compounds from *L. cambodiana*. This is the first report on chemical investigation of *L. cambodiana* species.

II. MATERIALS AND METHODS

A. *General experiment procedures*

NMR spectra were acquired on a Bruker AM500 FT-NMR spectrometer (Bruker BioSpin, Bremen, Germany) using TMS as an internal standard. Electrospray ionization mass spectra were recorded on an Agilent 1100 Mass spectrometer (Agilent technology, Santa Clara, CA, USA). Column chromatography was performed using silica gel (Merck, Whitehouse Station, NJ, USA) and reverse phase C18 resins (YMC Ltd., Kyoto, Japan). Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-C18 F_{254S} plates (0.25 mm, Merck). Spots were visualized under UV radiation (254 and 365 nm) and sprayed with aqueous solution of H_2SO_4 (10%), heating with a heat gun.

B. *Plant materials*

The leaves of *Lonicera cambodiana* Pierre ex Danguy were collected at Hoa Binh Province, Vietnam in June 2013. Its scientific name was identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Hanoi, Vietnam. A voucher specimen (MAI02-2013) is deposited at the Institute of Ecology and Biological Resources.

C. *Extraction and Isolation*

The dried powdered leaves of *L. cambodiana* (3.0 kg) was ultrasonically extracted in methanol (3 times, each 5L in 60 minutes). After removal of methanol *in vacuo*, a dark solid methanol extract (195 g) was obtained. This extract was then suspended in 1L of distilled water and separated with chloroform (1L) to give corresponding chloroform extract (50 g) and water layer (145 g). The water layer was passed through diaion HP-20 column chromatography (CC) and eluted with increasing methanol in water (0-100% volume of methanol) to give three fractions (W1-W3). Fraction W1 was repeatedly chromatographed on a silica gel column, eluting with gradient solvents of ethyl acetate and methanol (0-100% volume of methanol) to yield 10 fractions (W1.1-W1.10). Compounds **1** (20 mg) and **2** (6 mg) were obtained from fraction W1.2 using reverse phase C18 resins CC and methanol/water (2/1, v/v) as an eluent. Next, fraction W1.4 was subjected on a sephadex LH-20 CC and eluted with methanol/water (1/1, v/v) to yield two sub-fractions W1.4A and W1.4B. The W1.4B were purified on a silica gel CC, eluting with chloroform/methanol/water (5/1/0.1, v/v/v) to give compounds **3** (7.0 mg) and **4** (4.6 mg).

- Loganin (**1**): Molecular formula: $C_{17}H_{26}O_{10}$; White amorphous powder; $[\alpha]_D^{25}$ = -34.6 (c 0.07, MeOH); ESI-MS *m/z*: 391 $[M+H]^+$; ^{1}H and ^{13}C-NMR data are given in the Table I and Table II.

- Sweroside (**2**): Molecular formula: $C_{16}H_{22}O_9$; White amorphous powder, $[\alpha]_D^{25}$ = -26.1 (c 0.06, MeOH); ESI-MS *m/z*: 359 $[M+H]^+$; ^{1}H and ^{13}C-NMR data are given in the Table I and Table II.

- Pinoresinol 4-O-β-D-glucopyranoside (**3**): Molecular formula: $C_{26}H_{32}O_{11}$; Pale yellow amorphous powder; $[\alpha]_D^{25}$ = +36.1 (c 0.01, MeOH); ^{1}H and

TABLE I. ^{1}H-NMR DATA FOR COMPOUNDS **1-4**

Comp	**1**	**2**	**3**	**4**
	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
1/1a	5.28 (d, 4.5)	5.57 (br s)	-	-
2/2a	-	-	7.05/6.97 (d, 2.0)	6.73/6.67 (s)
3/3a	7.40 (br s)	7.62 (d, 2.0)	-	-
4/4a	-	-	-	-
5/5a	3.10 (m)	3.17 (m)	7.16/6.79 (d, 8.0)	-
6/6a	1.63 (m) 2.24 (m)	1.78 (m)	6.94/6.83 (dd, 2.0, 8.0)	6.73/6.67 (s)
7/7a	4.05 (m)	4.39 (m)	4.78/4.73 (d, 4.0)	4.77/4.72 (d, 4.0)
8/8a	1.88 (m)	5.58 (m)	3.15/3.15 (m)	3.20/3.18 (m)
9/9a	2.05 (m)	2.73 (m)	3.72/3.69 (dd, 5.0, 11.0) 4.26/4.26 (m)	3.91/3.89 (dd, 4.5, 11.0) 4.29/4.29 (m)
10	1.10 (d, 7.0)	5.30 (dd, 1.5, 10.0) 5.34 (dd, 1.5, 17.5)		
11	-	-		
1'	4.67 (d, 8.0)	4.71 (d, 8.0)	4.89 (d, 7.0)	4.87 (d, 7.5)
2'	3.31 (dd, 8.0, 9.0)	3.21 (dd, 8.0, 9.0)	3.50 (m)	3.49 (m)
3'	3.39 (t, 8.5)	3.41 (t, 9.0)	3.50 (m)	3.43 (m)
4'	3.32 (m)	3.34 (m)	3.50 (m)	3.43 (m)
5'	3.32 (m)	3.34 (m)	3.42 (m)	3.21 (m)
6'	3.67 (dd, 6.0, 11.5) 3.92 (dd, 2.0, 11.5)	3.69 (dd, 6.0, 12.0) 3.92 (dd, 2.0, 12.0)	3.70 (dd, 6.0, 11.5) 3.88*	3.67 (dd, 5.5, 12.0) 3.79 (dd, 2.5, 12.0)
OMe	3.70 (s)	-	3.89/3.87 (s)	3.86/3.85 (s)

*)Overlapped signals.

^{13}C-NMR data are given in the Table I and Table II.

- Syringaresinol 4-O-β-D-glucopyranoside (**4**): Molecular formula: $C_{28}H_{36}O_{13}$; Pale yellow amorphous powder; ^{1}H and ^{13}C-NMR data are given in the Table I and Table II.

III. RESULTS AND DISCUSSION

Dried leaves of *L. cambodiana* was extracted with methanol and separated with chloroform to give chloroform and water residues. Using various chromatographic technique, four compounds (**1-4**) were isolated from water residues of *L. cambodiana* leaves (Fig. 1).

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** showed proton signals characteristic for an olefinic proton at δ_H 7.40 (1H, br s), two hemiacetal protons at δ_H 5.28 (1H, d, J = 4.5 Hz) and 4.67 (1H, d, *J* = 8.0 Hz), a methoxy group at δ_H 3.70 (3H, s), and a methyl group at δ_H 1.10 (3H, d, *J* = 7.0 Hz). Analysis of ^{13}C-NMR spectrum of **1** revealed signals of 17 carbons which were divided by DEPT spectrum into two non-protonated carbons, eleven methins, two methylens, and two methyl. In detail, a set of six carbon signals including δ_C 99.9, 78.2, 77.9, 74.6, 71.5, 62.7 were charactesized for a glucose unit. A methoxy group was indicated by carbon signals at δ_C 51.6. Remaining 10 carbons were belonged an iridoid skeleton. Among them, carbon signals at δ_C 169.5 was assigned for a carboxylic carbon. A pair of olefinic carbon signals at δ_C 152.1 (CH) and 113.9 (C) were suggested for the presence of a double bond in the compound **1**. Furthermore, their chemical shift values (δ_C 152.1 and 113.9) indicated that this double bond located at C-3/C-4 of an iridoid skeleton and a methocarboxy group was at C-4. The doublet methyl proton signals at δ_H 1.10 and its corresponding carbon signal at δ_C 13.4 was assigned for H-10 and C-10 of and iridoid and hence the last oxygenated methin at δ_C 75.0 suggested location of hydroxyl group at C-6. From above evidence, compound **1** was deduced to be loganin. The NMR data of **1** was well agreed with those reported in the literature [5]. In addition, ESI-MS analysis of **1** was also suitable with a molecular formula of of loganin, $C_{17}H_{26}O_{10}$, by a quasi-molecular ion peak at *m/z* 391.

TABLE II. ^{13}C-NMR DATA FOR COMPOUNDS **1**-**4**

Comp	1	2	3	4
	δ_C	δ_C	δ_C	δ_C
1/1a	97.6	97.9	137.5/133.8	139.7/136.3
2/2a	-	-	111.7/111.0	105.0/104.7
3/3a	152.1	153.9	147.5/146.2	154.6/149.5
4/4a	113.9	106.0	151.0/148.2	135.8/133.2
5/5a	32.0	28.4	118.1/116.2	154.6/149.5
6/6a	42.1	25.8	120.0/119.8	154.6/149.5
7/7a	75.0	69.6	87.5/87.0	87.8/87.3
8/8a	42.6	133.3	55.5/55.3	55.9/55.7
9/9a	46.4	43.7	72.6/72.2	73.1/73.0
10	13.4	120.8	-	-
11	169.5	168.4	-	-
1'	99.9	99.7	102.8	105.5
2'	74.6	74.6	74.9	75.9
3'	77.9	78.3	77.8	78.0
4'	71.5	71.4	71.3	71.5
5'	78.2	77.8	78.2	78.5
6'	62.7	62.6	62.5	62.7
OMe	51.6	-	56.8/56.4	57.2/57.0

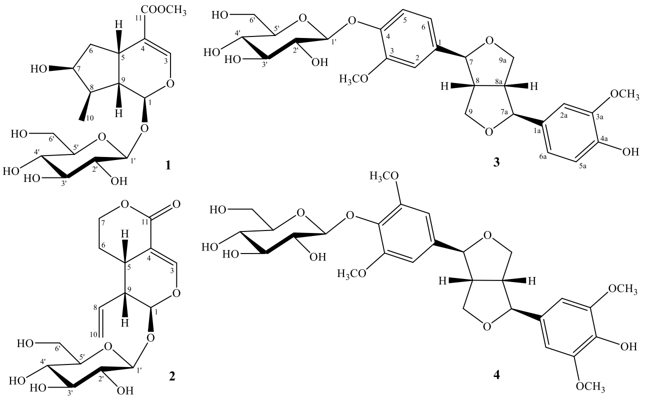

Fig. 1. *Chemical structures of compounds 1-4*

Compound **2** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of **2** observed signals of four olefinic protons including a vinyl group at δ_H 5.34 (1H, dd, *J* = 1.5, 17.5 Hz), 5.30 (1H, dd, *J* = 1.5, 10.0 Hz), 5.58 (1H, m); and another deshielded olefinic proton at δ_H 7.62 (1H, d, *J* = 2.0 Hz). The ^{13}C-NMR and DEPT spectra of **2** displayed signals of sixteen carbons with a carbonyl carbon δ_C 168.4; quaternary olefinic carbon at δ_C 106.0; a vinyl group at δ_C 120.8 and 133.3; two hemiacetal carbons δ_C 97.9 and 99.7. Like compound **1**, a set of carbon signals at δ_C 99.7, 78.3, 77.8, 74.6, 71.4, and 62.6 was indicated the presence of a glucose unit. Teen remaining carbons containing a vinyl group suggested that compound **2** belonged to a secoiridoid structure by opening five-membering of iridoid at C-7/C-8. In addition, the chemical shift of carbonyl carbon δ_C 168.4 (C-11) and oxygenated methylene carbon δ_C 69.6 (C-7) suggested a lactone formation between C-11 and C-7. Thus, chemical structure of **2** was suggested to be sweroside. Finally, NMR data of **2** were compared with those of sweroside in the literature and well matched each other [6]. The ESI-MS analysis of **2** was also consisted with sweroside molecular formula, $C_{16}H_{22}O_9$, showing quasi-molecular ion peak at m/z 359 $[M+H]^+$.

Compound **3** was obtained as a pale yellow amorphous powder. ^{1}H-NMR spectrum of **3** indicated signals of two ABX spin coupling system at δ_H 7.05/6.97 (each 1H, d, J = 2.0 Hz), 7.16/6.79 (each 1H, d, *J* = 8.0 Hz), and 6.94/6.83 (each 1H, dd, J = 2.0, 8.0 Hz). Analysis of ^{13}C-NMR spectra of compound **3** also revealed signals of a glucose unit at δ_C 102.8, 78.2, 77.8, 74.9, 71.3, and 62.5. Two methoxy groups were indicated by corresponding proton and carbon signals at δ_H 3.89/3.87 (each 3H, s) and δ_C 56.8/56.4. The eighteen remaining carbons dividing into two phenylpropanoid fragments (Table 2) suggested compound **3** to be a lignan. Furthermore, except surgar moiety, carbon and proton signals of aglycone appeared in a pair signals with slight difference, indicating a symetric structure of aglycone. Moreover, carbon chemical shifts of C-7/C-7a, C-8/C-8a, and C-9/C-9a at δ_C 87.5/87.0, 55.5/55.3, 72.6/72.2 suggesting for a 7-9a:9-7a diepoxylignan. Consequently, chemical structure of **3** was deduced to be pinoresinol 4-O-β-D-glucopyranoside. The NMR data of **3** were well consisted with those reported in the literature [7].

Compound **4** was also isolated as a pale yellow amorphous powder. The ^{13}C-NMR data of **4** at C-7/C-7a, C-8/C-8a, C-9/C-9a, and sugar moiety were quite identical with those of **3** indicated that both compounds **3** and **4** shared same back bone of a 7-9a:9-7a diepoxylignan glycoside. The difference between **4** and **3** were signals of aromatic regions. In the ^{1}H-NMR spectrum of **4** show a pair of singlet aromatic proton δ_H 6.73/6.67 (each 2H, s) and a pair of methoxy signals δ_H 3.86/3.85 (each 6H, s) suggested a pair of symmetric 1,3,4,5-tetrasubtituted benzene ring. Therefore, chemical structure of **4** was established to be syringaresinol 4-O-β-D-glucopyranoside and agreed with literature [8].

In conclusion, chemical study of the *L. cambodiana* was firstly carried out in this work and lead to the isolation of four compounds including a iridoid (loganin, **1**), a secoiridoid (sweroside, **2**), and two 7-9a:9-7a diepoxylignan glycoside (pinoresinol 4-O-β-D-glucopyranoside and syringaresinol 4-O-β-D-glucopyranoside, **3** and **4**, respectively). The chemical structures of isolated compounds were established with the aids of ESI-MS, NMR spectroscopic technique and comparison with literature.

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